A MULTIFUNCTIONAL CARBON NANOFIBER/BIOCERAMIC COATING FOR ORTHOPEDIC APPLICATIONS

[1]D. GOPI, [2]L. KAVITHA, [3]E. SHINYJOY, [4]N. MURUGAN, [5]M. SURENDIRAN

[1,3,4,5]Department of Chemistry, Periyar University, Salem 636 011, Tamilnadu, India
[1]Centre for Nanoscience and Nanotechnology, Periyar University, Salem 636 011, Tamilnadu, India
[2]Department of Physics, School of Basic and Applied Sciences, Central University of Tamilnadu, Thiruvarur-610 101, Tamilnadu, India
E-mail: [1]dhanaraj_gopi@yahoo.com

Abstract— Carbon nanofibers (CNF), one of the most significant nanomaterials with unique mechanical, biological and surface properties, has been shown as suitable for various biomedical applications. In the present work we report the development of CNF reinforced mineral (manganese) substituted hydroxyapatite (Mn-HAP) composite on titanium alloy (Ti6Al4V). Hydroxyapatite (HAP, $[Ca_{10}(PO_4)_6(OH)_2]$) is a widely used biomaterial for bone implant applications due to its osteoconductive and bioactive properties. Application of HAP in orthopedic implants suffers from its low fracture toughness and poor wear resistance. To surmount these issues, CNF is used as an excellent reinforcement material for imparting strength and toughness to brittle HAP. The phase composition and surface microstructure of the obtained coating were evaluated by FT-IR, XRD and SEM-EDX, respectively. The effect of reinforcement of CNF and the substitution of Mn in HAP on the mechanical and biological properties of the CNF/M-HAP coating was investigated. Also, the corrosion resistance of the composite coating on Ti6Al4V substrate in SBF was evaluated by potentiodynamic polarization studies. The cytotoxicity of the CNF/M-HAP composite coated Ti6Al4V was analyzed towards human osteoblast (HOS MG63) cells using MTT assay. The in-depth analysis of the results obtained in this study facilitates a better understanding of the CNF/Mn-HAP composite coating. The reinforcement of CNF in Mn-HAP could significantly enhance the adhesive strength of the coating. In addition, the substitution of Mn in HAP improves the biocompatibility of the coating which can be well evident from the cell culture results. Thus, the combination of mechanical property of CNF and the biocompatibility of Mn-HAP makes the CNF/M-HAP composite coating an ideal choice for orthopedic implant applications.

Index Terms— Carbon nanofiber, Manganese substituted hydroxyapatite, Orthopedic applications, Ti-6Al-4V.

I. INTRODUCTION

Various metallic implants such as 316L stainless steel (316L SS), cobalt-chromium (Co–Cr) and titanium (Ti) and its alloys, are used in orthopedic applications. Recently Ti and its alloys are getting much consideration for biomaterial implants because of their good corrosion resistance, high specific strength and biocompatibility [1,2]. Among the available metallic implants, Ti alloys are the most widely used orthopedic implants. However, its direct bone bonding ability in short time period is poor. Generally the ability of bone bonding with tissue can be improved by providing a bioactive ceramic coating on Ti alloys. Hydroxyapatite [HAP, $Ca_{10}(PO_4)_6(OH)_2$] is one of the phases of calcium phosphate that has been mainly known for its special ability to contact bone tissue. HAP exhibits excellent biocompatibility and bioactivity due to its chemical and structural similarities to bone and tooth minerals [3].

Furthermore, addition of bioactive ions into the HAP based bioceramic material might enhance the biological properties of the implants [4]. In particular, the favorable effect of bioactive ions such as magnesium (Mg^{2+}), strontium (Sr^{2+}), manganese (Mn^{2+}), silver (Ag^{2+}), zinc (Zn^{2+}), silicon (Si^{2+}) and carbonate (CO_3^{2-}) in HAP based coatings on metallic substrates has been reported [5]. Manganese is a trace element which is essential for the growth and development of bones. The radius of manganese ion (0.99 °A) is very close to that of calcium (0.90 °A), which enables Mn ion to enter osteoblasts through calcium ion channel. Mn^{2+} influences bone metabolism by regulating the osteoblast differentiation and bone resorption [6].

Though the bioactive HAP based coating on metallic materials is proven to be efficient for better biocompatibility, they possess brittle nature and poor strength which impedes its clinical applications under load-bearing conditions.

To overcome this problem, carbon nanofiber (CNF) with identical chemical structures to that of carbon nanotubes would be a prior choice [7]. As an ideal reinforcing material for HAP and also as a coating material for metallic implants, they have good biocompatibility and excellent mechanical properties matchable to those of natural bone. However, the reinforcement of CNF in Mn-HAP has not been reported.

A number of coating technologies are available for the development of bioceramic composite coatings and among them the electrochemical deposition has exceptional advantages like low deposition temperature, process simplicity, capability of forming a uniform coating on metallic substrates and the low cost of equipments. It is thus established as one of the

most promising methods for obtaining coating on Ti–6Al–4V alloy [8,9]. Recently Gopi et al., have succeeded in the electrochemical deposition of bioceramic coating on Ti–6Al–4V alloy and also on the other metallic materials [10-13]. To the best of authors' knowledge, there are no reports on the CNF reinforced Mn substituted HAP coating for improved mechanical, corrosion resistive and biological properties. Hence, the aim of this study is to develop CNF/Mn-HAP composite coating on Ti–6Al–4V alloy with enhanced corrosion resistance and biocompatibility for promising orthopedic applications.

II. EXPERIMENTAL

A. Materials

Commercially available calcium nitrate dihydrate ($Ca(NO_3)_2.2H_2O$), manganese nitrate hexahydrate ($Mn(NO_3)_2.6H_2O)),$), dipotassium hydrogen phosphate (K_2HPO_4), carbon nanofiber (CNF), nitric acid and sulphuric acid, purchased from Aldrich Chemical Co. (Aldrich, India), were used for the development of CNF/Mn-HAP composite coating on Ti-6Al-4V alloy by electrochemical deposition. All chemicals were of analytical grade and were used as received and deionized water was used throughout the experiment.

B. Oxidation of CNF

The surface treatment of CNF with acid, oxidizing agents and surfactants produces carboxylic, hydroxyl and ketonic groups on the surface which modify the properties of CNF and improves dispersion and interactions with HAP matrix [14]. The Functionalisation of CNF was carried out in a round-bottomed flask equipped with a reflux condenser and a thermometer. Three hundred mL of a concentrated nitric acid is added to 1 wt% CNF. The mixture is refluxed for 24 h at 70°C. After cooling to room temperature, the reaction mixture is diluted with 500 ml of deionised water and then vacuum-filtered through a filter paper.

C. Sample preparation

Commercially available Ti–6Al–4V alloy with chemical composition (wt.%): 5.7 Al, 3.85 V, 0.18 Fe, 0.038 C, 0.106 O, 0.035 N and balance titanium is used as a substrate in this present study. The specimens were cut into a size of $10 \times 10 \times 3$ mm^3 and then embedded in epoxy resin, leaving area of 1 cm^2 for exposure to the test solution. The samples were ground mechanically using different grades of SiC emery papers ranging from 600 to 1500 grit and the final polishing was done with diamond paste of 1.5 μm. The resultant specimens were ultrasonically cleaned in acetone, and dried with warm air.

D. Electrodeposition of CNF/Mn-HAP

The electrodeposition of CNF/Mn-HAP on Ti–6Al–4V alloy was performed in a conventional three electrode cell configuration using a CHI 760C electrochemical workstation (USA). The deposition was carried out in potentiostatic mode by applying a potential of -1.4 V vs. SCE during 1 h using an electrochemical system CHI 760C (CH instruments, USA). After the specimen coated with composite coating they were rinsed with deionised water and dried for 5 h

E. Surface Characterization of the CNF/Mn-HAP coating

The coated samples were characterized for their functional groups by Fourier transform infrared spectroscopy (FT-IR, Impact 400 D Nicholet Spectrometer) over the frequency range from 4000 to 400 cm^{-1} with a number of 32 scans and spectral resolution of 4 cm^{-1}. The phase compositions of the coatings were identified by X-ray diffraction (XRD, Seifert, X-ray diffractometer Siemens D500 Spectrometer) in the range between $20^o \leq 2\theta \leq 60^o$ with CuKα radiation generated at 40 kV and 30 mA with a step size of 0.02^o at a scanning rate of 10 min^{-1} with standard data compiled by the International Centre for Diffraction Data (ICDD). The surface morphology of the samples was studied using high resolution scanning electron microscopy (HRSEM, JEOL JSM-6400, Japan). The elemental composition of the samples was investigated by energy dispersive X-ray analysis (EDAX).

F. Adhesion Test

The Mn-HAP, and CNF/Mn-HAP coatings on Ti–6Al–4V alloy, respectively were evaluated for its adhesion strength by pull-out tests according to the American Society for Testing Materials (ASTM) international standard F 1044-05 with at least five measurements for each experiment. The coated samples were cured in an oven at 100 ^oC for 50 min and then were subjected to pull-out tests at a crosshead speed of 1 mm min^{-1}, using a universal testing machine (Model 5569, Instron) [15].

G. Electrochemical investigation of the coatings

Potentiodynamic polarisation was carried out in simulated body fluid (SBF) solution to analyse the corrosion protection performance of the uncoated, Mn-HAP coated, and CNF/Mn-HAP coated Ti–6Al–4V alloy samples, respectively.

The SBF solution was prepared in accordance with procedure adopted by Kokubo et al., [16] and the pH value of the SBF solution was maintained at 7.4 to mimic the concentration of human blood plasma. Generally, the electrochemical experiments were conducted using a typical three-electrode system (CHI 760C, USA), using SCE as the reference electrode, Ti–6Al–4V alloy samples as the working electrode and platinum electrode as the counter electrode. Potentiodynamic polarisation studies for the coatings on Ti–6Al–4V alloy was carried out at a scan rate of 1 mV/s with the potential beginning from -200 to 1200 mV vs. SCE.

H. In vitro cytotoxixity

Human osteosarcoma MG63 cells (HOS MG63, ATCC CRL-1427TM) supplied by National Centre for Cell Sciences (NCCS), Pune, India, were cultured in standard culture medium, Dulbecco's Modified Eagle Medium (DMEM, GIBCO). The medium was renewed for every 2 days and the cultures were maintained in a humidified atmosphere with 5% CO_2 and 95% humidified air, at 37 °C. The osteoblast cultures were then detached from the culture flask by incubation with 0.1% trypsin and 0.1% ethylene diamine tetra acetic acid (EDTA) for 5 min. The viability of cells colonizing on the samples were evaluated by measuring the mitochondrial dehydrogenase activity using a modified MTT 3-(4,5-dimetyl-2-tiazolyl)-2,5-diphenyl-2-tetrazolium bromide) assay. To determine the cytotoxicity of the coated samples, HOS MG63 cells were seeded in 12-well plates at 104 cells/mL. After 24 h of incubation, MTT solution in 1 mL serum free medium was added and incubated for 4 h at 37 °C in a humidified 5% CO_2 atmosphere. To determine the cytotoxicity, an MTT assay was examined as a function of incubation time for 1, 4 and 7 days. The solution was then removed, dimethyl sulfoxide was added to it, and the plate was shaken for 15 min before measuring absorbance at 570 nm on an ELISA microplate reader and then % cell viability was calculated with respect to control as follows,

% Cell viability = [A] Test / [A] Control x 100

III. RESULTS AND DISCUSSION

A. FT-IR Analysis

The spectrum corresponding to the CNF/Mn-HAP as shown in Fig. 1 exhibits a structure similar to that for the CNF/HAP which indicates that the ionic radius of Mn (0.90 nm) is smaller than that of calcium (0.100 nm) [17].

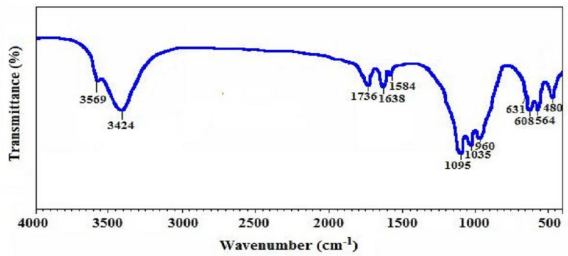


Fig. 1. FTIR spectrum of CNF/Mn-HAP coating.

The fundamental vibrational modes of the phosphate (PO_4^{3-}) peaks appeared at 1095 and 1035 cm^{-1} (υ_3), 608 and 564 cm^{-1} (υ_4), 480 cm^{-1} (υ_2) and 960 cm^{-1} (υ_1), respectively. The peaks observed at 3424 cm^{-1} and 631 cm^{-1} are assigned to the stretching and bending vibration of the hydroxyl group and peaks at 3424 cm^{-1} and 1638 cm^{-1} correspond to stretching and bending modes of water molecule of HAP, respectively. In addition, the peaks at 1736 cm^{-1} and 1584 cm^{-1} were found which is corresponding to the carboxyl group and carboxylate group on the surface of CNF obtained as a result of functionalization of CNF [18,19]. Thus, the FTIR spectrum suggests the formation of CNF/Mn-HAP coating.

B. XRD Analysis

The XRD pattern of CNF/Mn-HAP composite coated Ti–6Al–4V alloy is shown in Fig. 2. The diffraction peaks close to 26° can be assigned to the diffraction of (0 0 2) planes of graphite (ICDD card number: 13-0148). This result indicates the presence of that the graphitic structure of CNF in the CNF/Mn-HAP coating. At the same time, the peaks located at 26°, 31°, 33°, 41°, 45° and 51° can be indexed to the (0 0 2), (2 1 1), (3 0 0), (1 0 3), (2 0 3), (4 1 0) and planes of HAP (ICDD card number: 09-0432). This result demonstrates the successful formation of the CNF/Mn-HAP composite coating.

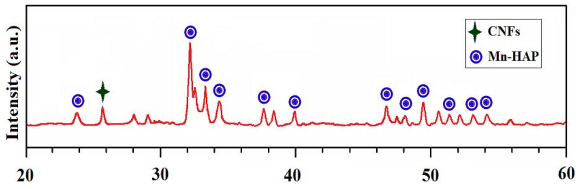


Fig. 2 XRD pattern for CNF/Mn-HAP coating.

C. SEM and EDAX Analysis

HRSEM micrograph of the CNF/Mn-HAP coated Ti–6Al–4V alloy is shown in Fig. 3a. The surface morphological evaluation of the CNF/Mn-HAP coating exhibited a complete surface coverage and homogeneous arrangement of CNF/Mn-HAP. From the figure it can be evident that the interconnected network like CNF/Mn-HAP structure can provide a significant increase in the mechanical strength of the coated substrate. Fig. 3b, shows the EDAX spectrum of the CNF/Mn-HAP composite coated Ti–6Al–4V alloy which indicates the presence of Ca, P and Mn along with the peaks for C. This result supports for the formation of CNF/Mn-HAP coating on Ti–6Al–4V alloy.

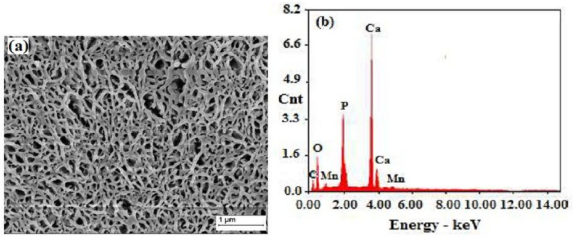


Fig. 3 (a) SEM image and (b) EDAX analysis of CNF/Mn-HAP coating.

D. Adhesion Strength

The adhesion strength of CNF/Mn-HAP coating on Ti–6Al–4V alloy specimen is one of the most important properties for bone implant application.

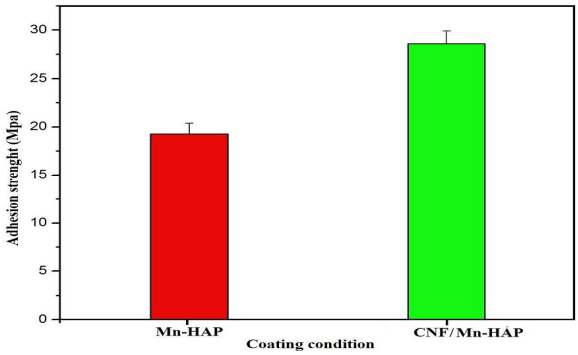


Fig. 4. Adhesion strength of Mn-HAP and CNF/Mn-HAP coating.

Fig. 4 shows the adhesion strength of Mn-HAP and CNF/Mn-HAP coatings on Ti–6Al–4V alloy, respectively. The adhesion strength of Mn-HAP and CNF/Mn-HAP composite coating on Ti–6Al–4V alloy was of 19.3±1.0, and 26.6±1.3, respectively. Thus, the as-developed CNF/Mn-HAP coated Ti-6Al-4V with adhesion strength of 26.6±1.3 will be suitable for orthopedic applications.

E. Potentiodynamic polarization studies

Fig. 5 shows the potentiodynamic polarization curves of the uncoated, Mn-HAP coated, and CNF/Mn-HAP coated Ti-6Al-4V alloy, respectively in SBF solution. From the figure, the electrochemical polarization parameters such as corrosion potential (E_{corr}) and corrosion current density (I_{corr}) were calculated. The polarization results of the uncoated Ti-6Al-4V alloy revealed the E_{corr} and I_{corr} values as -10 mV vs. SCE and 1.22 μA/cm^2, respectively. While the polarization curves of the Mn-HAP coating on Ti-6Al-4V alloy sample show the E_{corr} and I_{corr} values as 217 mV vs. SCE and 0.57 μA/cm^2, respectively. The potentiodynamic polarisation curve recorded for CNF/Mn-HAP coated Ti-6Al-4V alloy sample showed the E_{corr} and I_{corr} values as 300 mV vs. SCE and 0.35 μA/cm^2, respectively. Thus, from the obtained results it can be evident that, the polarization values obtained for CNF/Mn-HAP coated Ti-6Al-4V alloy was found to be nobler than that of the Mn-HAP coated and uncoated Ti-6Al-4V alloy which is evident for the corrosion resistance property of CNF. Thus, the CNF/Mn-HAP coated Ti-6Al-4V can be utilized in load bearing applications.

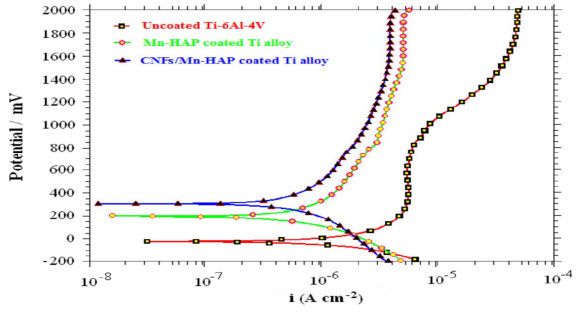


Fig. 5. Potentiodynamic polarization curves for uncoated, Mn-HAP and CNF/Mn-HAP coated Ti-6Al-4V.

F. In Vitro cytotoxicity study with HOS MG63 Cells

The % cell viability was calculated for CNF/Mn-HAP coating on Ti–6Al–4V alloy and was compared with the control. The viability of the cells grown on the coatings at 1, 4 and 7 days of culture is shown in Fig. 6. The CNF/Mn-HAP coating exhibited an extensive cell viability at day 7. The cell viability of the CNF/Mn-HAP coating is mainly due to the presence of Mn mineral substituted in HAP and also indicates that the CNF were non toxic. Thus, from the MTT assay test it is clear that CNF/Mn-HAP coated Ti-6Al-4V can be utilized for orthopedic applications.

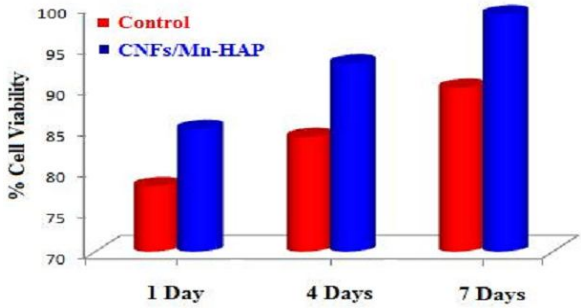


Fig. 6. % Cell viability of CNF/Mn-HAP coating at different days of incubation.

CONCLUSIONS

In this paper, we have demonstrated the effectiveness of mechanical, corrosion protection and bioactive behavior of electrodeposited CNF/Mn-HAP coating on Ti-6Al-4V alloy. The FT-IR, XRD, SEM-EDAX confirmed the formation of CNF/Mn-HAP coating on Ti-6Al-4V. The adhesive strength of CNF reinforced Mn-HAP coating is increased with the incorporation of CNF. The potentiodynamic polarization showed more favorable values for the CNF/Mn-HAP composite coated Ti-6Al-4V which implies that the corrosion resistance of the Ti-6Al-4V was significantly improved by the development of CNF/Mn-HAP composite coating on it. Also, the CNF/M-HAP composite coating showed better viability for HOS MG63 cells which enlightened the bioactive behavior of the coating. These multidirectional results indicate that the CNF/M-HAP composite coating on Ti-6Al-4V could act as a prospective implant material for orthopedic applications with improved mechanical, corrosion resistance and biocompatible properties.

ACKNOWLEDGEMENT

D. Gopi acknowledges the major financial support from the Department of Science and Technology (DST), and Council of Scientific and Industrial Research (CSIR), New Delhi, India. Also, D. Gopi acknowledges the University Grants Commission (UGC), New Delhi, India for the Research Award.

REFERENCES

[1] Z.Cai, H. Nakajima, M. Wold, A. Berglund, M. Bergman, T.Okabe, In vitro corrosion resistance of titanium made using different fabrication methods, Biomaterials, vol. 20, pp. 183-190, 1999.

[2] D. Gopi, A. Karthika, D. Rajeswari, L. Kavitha, R. Pramod, J. Dwivedi, Investigation on corrosion protection and mechanical performance of minerals substituted hydroxyapatite coating on HELCDEB-treated titanium using pulsed electrodeposition method, RSC Adv., Vol. 4, pp. 34751-34759, 2014.

[3] D. Gopi, E. Shinyjoy, M. Sekar, M. Surendiran, L. Kavitha, T.S. Sampath Kumar, Development of carbon nanotubes reinforced hydroxapatite composite coatings on titanium by electrodeposition method, Corros. Sci., vol. 73, pp. 321-330, 2013.

[4] L. Yang, S.J. Amodio, F.Y.F. Barrere-deGroot, V. Everts, C.A. van Blitterswijk, P.Habibovic, The effects of inorganic additives to calcium phosphate on in vitro behavior of osteoblasts and osteoclasts, Biomaterials, vol. 31, pp. 2976-2989, 2010.

[5] D. Gopi, E. Shinyjoy and L. Kavitha, Influence of ionic substitution in improving the biological property of carbon nanotubes reinforced hydroxyapatite composite coating on titanium for orthopedic applications, Ceram. Int., vol. 41, pp. 5454–5463, 2015.

[6] Y.T. Liu, K.C. Kung, T.M. Lee, T.S. Lui, Enhancing biological properties of porous coatings through the incorporation of manganese, J. Alloys Compd., vol. 581, pp. 459−467, 2013.

[7] Z. Ryu, H. Rong, J. Zheng, M. Wang, B. Zhang, Microstructure and chemical analysis of PAN-based activated carbon fibers prepared by different activation methods, Carbon, vol. 40, pp. 1144–1147, 2002.

[8] Y. Song, S. Zhang, J. Li, C. Zhao, X. Zhang, Electrodeposition of Ca–P coatings on biodegradable Mg alloy: invitro biomineralization behavior, Acta Biomater., vol. 6, pp. 1736–1742, 2010.

[9] W.V. Raemdonk, P. Ducheyne, P.D. Meester, Structural analysis of hydroxyapatite coating on titanium, J.Am.Ceram.Soc., vol. 63, pp. 381–384, 1984.

[10] D. Gopi, El-Sayed M. Sherif, D. Rajeshwari, L. Kavitha, R. Pramod, Jishnu Dwivedi, S.R. Polaki, Evaluation of the mechanical and corrosion protection performances of electrodeposited hydroxyapatite on the high energy electron beam treated titanium alloy, J. alloys cmds., vol. 616, pp. 498-504, 2014.

[11] D. Gopi, A. Karthika, S. Nithiya and L. Kavitha, In vitro biological performance of minerals substituted hydroxyapatite coating by pulsed electrodeposition method, Mater. Chem. Phys., vol. 144, pp. 75-85, 2014.

[12] D. Rajeswari, D. Gopi, S. Ramya and L. Kavitha, Investigation of anticorrosive, antibacterial and in-vitro biological properties of sulphonated poly(etheretherketone)/strontium, cerium co-substituted hydroxyapatite composite coating developed on surface treated surgical grade stainless steel for orthopedic applications, RSC Adv., vol. 4, pp. 61525-61536, 2014.

[13] D. Gopi, N. Murugan, S. Ramya, E. Shinyjoy, L. Kavitha, Ball flower like manganese, strontium substituted hydroxyapatite/cerium oxide dual coating on AZ91 Mg alloy with improved bioactive and corrosion resistance properties for implant applications, RSC Adv., vol. 5, pp. 27402-27411, 2015,

[14] P.A. Tran, L. Zhang, T.J. Webster, Carbon nanofibers and carbon nanotubes in regenerative medicine, Adv. Drug Delivery Rev., vol. 61 pp. 1097–1114, 2009.

[15] ASTM standard F 1044-05, ASTM International, West Conshohocken, PA.

[16] T. Kokubo, H. Takafama, How useful is SBF in predicting in vivo bone bioactivity?, Biomaterials, vol. 27, pp. 2907–2915, 2006.

[17] K. Cui, Y. Song, Q. Guo, F. Xu, Y. Zhang, Y. Shi, L. Wang, H. Hou, Z. Li , Architecture of electrospun carbon anofibers–hydroxyapatite composite and its application act as a platform in biosensing, Sensors and Actuators B, vol. 160, pp. 435–440, 2011.

[18] L.N. Wu, X.J. Zhang, H.X. Ju, Amperometric glucose sensor based on catalytic reduction of dissolved oxygen at soluble carbon nanofiber, Biosens. Bioelectron., vol. 23, pp. 479-484, 2007.

[19] J. Chem, M.A. Hamon, H. Hu, Y. Chen, A.M. Rao, P.C. Eklund, R.C. Haddon, Solution properties of single-walled carbon nanotubes, Science, vol. 282, pp. 95-98, 1998.

★★★